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(57) Abstract

Peptides of the alpha subunit of Class II MHC antigens are employed for modulation of T-cell activity. The peptides can be used in therapies, particularly associated with transplantation, by themselves or in conjunction with other agents.

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IMMUNE MODULATION WITH CLASS II ALPHA-CHAIN FRAGMENTS

INTRODUCTION

Technical Field

The field of this invention is immunomodulating therapies.

Background

Despite advances in immunosuppressive therapy, the major barrier to successful transplant engraftment is the allogeneic immune response which results in graft rejection or graft vs. host disease. Currently available non-specific immunosuppressive therapies are accompanied by increased risks of infection and a variety of deleterious side effects, including nephrotoxicity, hypertension, hyperlipidemia and bone disease. Even with the current armamentarium of immunosuppressive agents, acute graft rejection and failure to achieve long-lasting graft survival persists.

The humoral and cellular lymphoid responses are slowly being elucidated. However, despite the substantial progress which has been achieved, there still remains numerous aspects of the immune response which have eluded explanation. One aspect which has substantial significance for successful transplanting is tolerization, which may be associated with cell depletion, suppression, or anergy.

Besides transplants, there are other indications where suppression of T cell proliferation and/or activation would be of interest. These include autoimmunity, cancer, T cell mediated cytotoxicity, or the like. There would therefore be a substantial value to be able to enlist naturally occurring processes associated with tolerization to provide for acceptance of allogeneic implants.

Sayegh *et al.*, Induction of Immunity and Oral Tolerance with Polymorphic Class II Major Histocompatibility Complex Allopeptides in the Rat, *Proc. Natl. Acad Sci. USA*, **89**:7762-7766 (1992). Sayegh *et al.*, Thymic Recognition of Class II Major Histocompatibility Complex Allopeptides Induces Donor Specific Unresponsiveness to Renal Allografts, *Transplantation* **56**:461 (1993). Sayegh *et al.*, Induction of Immunity and Oral Tolerance to Alloantigen by Polymorphic Class II Major Histocompatibility Complex Allopeptides in the Rat, *Transplantation Proc*: **25**:357 (1993). Beuichon *et al.*, Donor MHC Peptides Are Presented by Recepient MHC Molecules during Graft Rejection, *J. Exp. Med.* **175**:305 (1992). Wachtsinger *et al.*, Mechanisms of Allo-Recognition, *Transplantation* **57**:572-576 (1994).

SUMMARY OF THE INVENTION

Peptides corresponding to at least a portion of the α_1 helix of the alpha chain of Class II major histocompatibility complex antigens are employed for immunomodulation. The peptides, mutant derivatives thereof, and peptidomimetric agents are administered to a host where the immune response is to be modulated.

DESCRIPTION OF SPECIFIC EMBODIMENTS

In accordance with the subject invention, compounds, particularly oligopeptides are provided having the same sequence, or being able to compete with a sequence, of at least 8 amino acids coming within the α_1 helix of the alpha chain of a Class II major histocompatibility complex antigen, more particularly a human lymphocyte antigen (HLA). The Class II HLAs associated with human MHC antigens, or their equivalents in other species, include DP, DQ and DR. Of particular interest is the domain of the alpha chain comprising the amino acids from about amino acid 50 to amino acid 85, more usually from about amino acid 53 to 80, and particularly comprising the amino acids in the range from about71 to 80. While substantially longer amino acid sequences may be employed, these additional flanking amino acids groups will serve specific purposes, rather than providing the immunomodulation activity.

substituted, particularly with conservative substitutions. Furthermore, the entire α_1 helix does not seem to be essential for binding, so that only conserved regions among the oligopeptides are required for the desired effect. Even in the conserved regions, various substitutions may be made, where polarity and size variations may be accommodated by the binding partner. Conveniently substitutions may be made with alanine and valine, or the alternative amino acid, among the acidic, basic and amio acids, *i.e.* N and Q.

For the most part, the peptide compounds of this invention will include an oligopeptide of at least about 8 amino acids, coming within the following sequence:

Formula 1:

aa^{53} aa^{54} aa^{55} aa^{56} F aa^{58} aa^{59} Q aa^{61} aa^{62} L aa^{64} N I A
aa^{68} aa^{69} aa^{70} aa^{71} N L aa^{74} aa^{75} aa^{76} aa^{77} aa^{78} R aa^{80} aa^{81}
aa^{82} aa^{83}

wherein:

aa^{53} , aa^{54} and aa^{55} may be any amino acid, including neutral amino acids other than proline, both aliphatic and aromatic, acidic amino acids, or basic amino acids, more particularly V, I, L , D, E, K, R or F;

aa^{56} is K, R, S or T, particularly R or S;

aa^{58} is D or E;

aa^{59} is G, A or P, particularly A or P;

aa^{61} is A, G or F, particularly G or F;

aa^{62} is A or G;

aa^{64} is G, A, S or T, particularly A or T;

aa^{68} is I, L or V, particularly I or V;

aa^{69} is D, E, I, L or V, particularly D or L;

aa^{70} is K, N, Q or R, particularly K or N;

aa^{71} is H, N, Q, S or T, particularly H, N or S;

aa^{74} is D, E, N or Q, particularly E or N;

aa^{75} is I, L, S, T or V, particularly I or T;

aa^{76} is I, L, N, or V, particularly L, M or V;

aa^{77} is I, L, S, T or V, particularly I or T;

aa^{80} is S, T or Y, particularly S or Y;

aa^{81} is N, Q, V, A or Y;

aa^{82} is S, T, or C; and

aa^{83} is S, T, K or R

Of particular interest is a sequence having at least 8 amino acids coming within the sequence 71-79, where these amino acids in particular will be more subject to reduction in activity by replacement, although even in this region, some replacement will be permissible without total loss of activity.

There will generally be fewer than 20%, more usually fewer than 10% of the amino acids substituted or deleted, the number of substitutions usually not exceeding three, more usually not exceeding two. For the most part, these substitutions will be conservative substitutions, where the following table indicates that amino acids on the same line may be substituted for one another.

Table 1:

Aliphatic

non-polar

A, G (P)

I, L, V

polar (neutral)

S, T, M

N, Q

polar (charged)

D, E

K, R

Aromatic

F, H, W, Y

where P will usually not be used as a substitute for G or A, while I, L, M, N, Q and V may be substituted one for the other.

Table 2 :

DQ 03011	
(53-55)	
R F R	
(56-80)	
R F D P Q F A L T N I A V L K H N L N I V I K R S	
DP 0101	
(53-77)	
S - E A - G G - A - - - I - N N - - - T L - Q - -	
DR 0101	
(53-77)	
S - E A - G - - A - - - - D - S - - E - M T - - -	
DQ 010101	
(71-80)	
- - - - - M - - Y	

The subject peptides may be modified in a wide variety of ways. As already indicated, the subject peptides may be mutagenized, by employing natural or unnatural amino acids, particularly the D-stereoisomer. While for substitutions and deletions, usually the restrictions indicated previously will apply, where the substitution involves the different stereoisomer, all of the amino acids may be substituted with the unnatural stereoisomer. Sites 74and 77permit modification with retention of activity. For example, replacement of I with a neutral amino acid having about the same chain length (including heteroatoms, in the range of about 5 to 8), e.g. T, leads to only a partial loss of activity, while replacement of neutral N at position 74had substantially no effect on activity, where an acidic amino acid, D, was used as the replacement. By contrast, replacement of N with D abrogated the activity. The activity was inhibition of Conconavalin A induced T cell proliferation by the peptide. In order to determine whether a particular site is amenable to

combinatorial approaches, where the different peptides may be screened for their activity in the indicated assay. The assays are straight forward and the peptides may be screened for activity, where conservative and non-conservative substitutions may be employed to detrmine whether an amino acid at a particular site is essential. Usually, the number of substitutions will not exceed 3, more usually not exceed 2, and generally not exceed 1. When a particular site has been has determined not essential for activity, that site may be substituted, while substituting other sites to determine the effect of double substitution involving one non-essential site.

The subject peptides may be obtained from any mammalian source: such as domestic or laboratory animals; e.g. murine, bovine, canine, feline, equine, lagomorpha, primate; and particularly human. One may use allogeneic or xenogeneic sequences, since the subject sequences have activity across a wide range of different MHC antigen alleles.

In addition, the peptides may be joined by covalent bonds at any convenient site along the peptide to a variety of other compounds for different purposes, particularly at the termini, where the N-amino group may be substituted and/or the C-carboxyl group may be substituted.

The peptides may be joined to: a) immunogens for administration to a host for immunization for production of antibodiesl; b) a non-adjacent MHC sequence of the particular MHC antigen by means of synthesis, expression of a synthetic gene, or the like; c) a lipid or polyalkyleneoxy group; d) a sugar; or e) a nucleic acid. Of particular interest is joining the subject peptides to another peptide by synthesis or expression of a synthetic gene, where the other peptide provides for enhanced stability of the subject peptides when administered to a host. Various peptides may be used, such as the immunoglobulin constant region IgG Fc. The peptides may be linked to various labels, either directly or indirectly, for detection of binding of the peptides to a target molecule. Labels include enzymes, fluorescers, chemiluminescers, radioisotopes, or the like. In addition, the subject peptides may be bound to a solid substrate, such as particles, container walls, or the like, to serve for affinity separation of cells or cell fragments comprising molecules which have a specific affinity for the subject molecules.

mechansisms associated with T cell activation and anergy.The subvject peptides may be used toprevent activation of T cells to a particular stimulation, e.g. Concanavalin A, whereby the block in the pathway may be investigated. By using different agents, either intracellular or extracellular in conjunctionwith the subject peptides, one can isolate the pathway associated with the activation of T cells as influenced by the presence of the subject peptides. The subject peptides may be used in screening compounds as to their ability to override the effects of the subject peptides, to augment the effect of the subject peptides or to direct the T cell into an alternative pathway.

Subsets of T cells can be classified by their binding affinity to the subject peptides, as well as their other phenotypic characteristics. For example, tumor infiltrating lymphocytes, natural killer cells, cells that home to synovia, mucosal tissue, subcutaneous tissue, lymphoid tissue, or the like, can be classified as to their binding to the subject peptides. In this way, the subject peptides may be used for specific applications associated with specific indications.

The subject peptides may be joined together to provide a single polypeptide, where the polypeptide is a multimer of the single peptide, or combination of different subject peptides. Usually the number of sequences will be at least 2 and not more than 20, usually not more than about 10 sequences or repeats. Alternatively, the subject peptides may be combined in a mixture to provide for a broader spectrum of activities or synergistic activity.

The subject peptides may be prepared in a variety of ways. Conveniently they can be synthesized by conventional techniques employing automatic synthesizers, such as the Beckman, Applied Biosystem Inc., or other automatic apparatus, or may be synthesized manually. Alternatively, DNA sequences may be prepared which encode the particular peptide and may be cloned and expressed to provide the desired peptide. In this instance, methionine may be the first amino acid, or a signal sequence may be provided, whereby the expressed peptide is secreted with processing and removal of the signal peptide. Techniques for preparing synthesized DNA sequences are extensively described in the literature and

Sambrook *et al.*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY 1989.

Alternatively, the peptides may be isolated from natural sources and purified by known techniques, including, for example, chromatography on ion exchange materials, separation by size, immunoaffinity chromatography and electrophoresis. As used herein, the term "a substantially pure preparation of peptide compound" means a preparation of the peptide which is usually greater than about 70% free of materials with which the polypeptide is naturally associated, normally the natural or production source of the peptide, preferably greater than about 80% free of these materials, more preferably greater than about 95% free of these materials. These materials, however, exclude materials with which the peptide may be mixed in the preparation of pharmaceutical compositions.

The sequences may be modified in accordance with their intended purpose. Different N- or C-terminal groups may be introduced which allow for linking of the peptide to a solid substrate or other molecule. In a synthetic procedure for producing the peptides, any molecule may be introduced at an internal site or at a terminus, which may allow for subsequent reaction, depending upon the purpose for which the peptide is prepared.

For diagnostic purposes, a wide variety of labels may be linked to the terminus, which may provide, directly or indirectly, a detectable signal. For example, fluorescers may be introduced at the terminus or other molecules which provide a linkage to labels such as fluorescers, enzymes, particles, or the like, where the other molecules are haptenic or antigenic members of a specific binding pair or receptors, *e.g.* antibodies. For example, linkage may be introduced at the terminus, *e.g.*, biotin, which will bind to an avidin conjugate with enzymes or fluorescers. Alternatively, various reactive sites may be introduced at the terminus for linking to particles, solid substrates, macromolecules, or the like. For example, an internal amino moiety of a growing chain bound to a solid substrate with the intermediate side groups protected, may be conjugated with methyldithiobenzoic acid (MDTB). The free mercaptan group may then be used for conjugating with activated olefins. Thus, proteins, such as serum albumin, keyhole limpet hemocyanin, bovine β-globulin, or the like, may be conjugated to the peptide to provide for an

affinity chromatography, or the like. Alternatively, the peptide can be bonded to another polypeptide by preparing a DNA sequence which has the peptide at the N-terminus, C-terminus or internal to the protein, so as to provide a fused protein which includes the binding peptide of interest. In this manner, fused proteins may be produced which have enzymatic activity, which enzymatic activity may be modulated by macromolecules, *e.g.*, antibodies, binding to the peptide of interest. Thus, the peptides of the subject invention may be modified in a wide variety of ways for a variety of end purposes while still retaining biological activity.

By identifying T cells which bind to the subject peptides, whose activity is modulated by the subject peptides, one may determine whether one can contrl rejection in the case of transplants. By screening the recipient's T cells for binding and response to the subject peptides, one can define a course of therapy for preventing rejection.

Various techniques are available for joining a peptide or protein to a lipid, particularly a phospholipid to provide for the presence of the peptide or protein on a liposome or membrane surface. Phosphatidyl choline, phosphatidyl ethanolamine, or other lipid may be used with a bifunctional linking agent, such as MBSE, glutaraldehyde, methyldithiobenzoic acid, or the like. The formation of liposomes with conjugated proteins finds ample support in the literature, See, U.S. Patent Nos. 3,887,698; 4,261,975 and 4,193,983. Alternatively, one may provide for a coding sequence which joins the peptide to phosphatidyl inositol, farnesyl, geranylgeranyl, or myristoyl. The modified peptide or protein is combined with the lipids in an aqueous medium and sonicated to provide the desired liposomes. The liposomes may then be harvested and used in the ways indicated.

The subject peptides, by themselves, or in combination with other peptides or proteins, may be used for diagnosing the presence of CTLs which bind to a subject peptide or the combination of a subject peptide and other peptide or protein. Conjugates of the subject peptide and the other peptide or protein can be prepared by employing linking agents as described previously, where the other peptide or protein may be an enzyme to provide a detectable signal, an antigen for binding to an antibody for separation of binding CTLs. Alternatively, the subject peptide and the

or the like, covalently or through antibody/antigen binding. If desired, the subject peptide and antigenic peptide or protein may be conjugated to a particle or protein which is fluorescent. The binding of the particle or protein will allow for sorting and counting in a fluorescence activated cell sorter.

The subject peptides may also be used for modulating CTL activity in the mammalian host. The modulation may be by inhibiting CTL activity or by sensitizing target cells. This can be achieved by employing apheresis, *ex vivo*, where the patient's blood is withdrawn from the patient and circulated through a device in which the peptide is present, either bound to the surface to remove CTLs active with the subject peptide or by adding the peptide in a physiologically acceptable medium to bind to the CTLs and inhibit their activity. Alternatively, the subject peptides may be administered to a host intravascularly, in either an artery or vein, or at the site of the implant to provide for inhibition or stimulation of the CTL.

The subject peptide may be combined with a population of cells taken from a patient, where the cells are only a portion of the total cells in the periphery, or where the population is enriched for a particular cell type, *e.g.* CTLs, Class I T-cells, Class II T-cells, NK-cells and the like. Where the population is removed from the patient, the cells may be incubated for sufficient time for the cellular activity to be modulated. The cells may then be washed free of the peptide and restored to the host.

In the case of an organ implant the subject peptides may be administered prior to the implantation, usually not exceeding about two weeks prior and repetitive treatment may be employed. At or about the time of implantation the subject compound may be administered during or immediately prior to the operation. The subject peptides may be used to treat the organ, by bathing the organ in a medium containing the subject peptides. In this way CTLs present with the organ may be deactivated to prevent graft vs. host disease. The peptide may be present at a concentration of 10^{-3} to 10^{-6}M. The organ may be washed free of the storage medium or the organ used with the peptide bound to the cells of the organ.

The subject peptides or compounds comprising the subject peptides can inhibit CTL activity, thus preventing the lysis of target cells. In addition, the

differentiation of inactive precursors into active mature CTLs. Also, the subject peptides may be used to reduce the mitogenic response of CTLs to mitogens or other activating compounds, *e.g.* Concanavalin A or anti-CD3 antibody.

The subject peptides are found to be active with CTLs having a wide variety of MHC antigens, rather than being specific for the particular HLA antigen from which the subject peptides are derived.

The subject peptides may be formulated in a variety of ways for administration to a host. The subject peptides may be used by themselves or in combination with other immunosuppressant agents, such as cyclosporine A, FK506, immunotoxins, anti-OKT3 or the like. By employing the subject peptides in conjunction with known immunosuppressants, substantially reduced concentrations of the immunosuppressant may be used in the formulations, usually less than about 60%, more usually less than about 40% of the normal dosage employed in a particular situation, to provide sufficient inhibition of CTL activity. In this way, the substantial side effects of the immunosuppressant compositions may be ameliorated by virtue of the lower concentration. The subject agents may be used with peptides derived from the α helix of the α chain of Class I MHC antigens. See particularly, PCT\US93\01758 and references cited therein. Of particular interest is the peptide B2702.75-84, which may be present in one or more copies, particularly where there is an inverted repeat, e.g. 84-75/75-84.

Various physiologically acceptable media may be used in the formulations comprising the subject peptides, by themselves or in conjunction with other drugs, such as deionized water, saline, phosphate buffered saline, aqueous ethanol, etc. The concentration of the peptide may vary widely, since for the most part, the peptides will be highly soluble in the media. Usually, the peptide will be not more than about 75 weight-percent, more usually not more than about 50 weight-percent, and usually greater than about 1 weight-percent. The dosage will vary depending upon the purpose of the administration, the frequency of administration, the efficacy of the peptide, and the like. Generally, the dosage will range from about 0.01 to 10 mg/kg of host, based on active sequence.

encapsulated, introduced into the lumen of liposomes, prepared as a colloid, or other techniques may be employed which extend the lifetime of the peptides.

The subject peptides may be used in assays to evaluate the activity of agents in mimicking the activity of the subject peptides. One can use labeled peptides under binding conditions in competition with the agent in binding assays, with CTLs, microsomes, antibodies binding to the peptides, and the like. Thus, compounds may be screened for their effectiveness in comparison with the subject peptides and related to the mode of action provided by the subject peptides. The subject peptides may be used to standardize assay protocols for use in screening agents.

The subject peptides may also be used for detecting complementary compounds which bind to the subject peptides. In this way proteins which bind to the subject peptides may be isolated and purified, *e.g.* affinity chromatography.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Example 1: Preparation of peptides from Class II alpha chain.

Four peptides were prepared by conventional synthetic methods using standard solid-phase techniques. See Erickson and Merrifield in: The Proteins, Vol. 2, 3rd ed., eds. Neurath, H. and Hill, R.L., p. 255-527, Academic Press, N.Y. (1970), which is incorporated herein by reference. Three of the peptides had amino acids from the α_1 domain of amino acids 53-77, one had amino acids 56-80 of the α_1

the following compositions and designations:

```
DQ 03011     56-80
R F D P Q F A L T N I A V L K H N L N I V I K R S

DP 0101      53-77
S - E A - G G - A - - - I - N N - - - T L - Q - -

DR 0101      53-77
S - E A - G - - A - - - - D - S - - E - M T - - -

DQ 010101    71-80
                                    - - - - - M - - Y
```

Example 2: Peptides corresponding to residues 50-80 of the α chain of class II HLA block the differentiation of cytotoxic T lymphocytes (CTL) precursors into effector CTL.

PBL from normal donors were isolated over Ficoll and cultured in 24 replicates at the indicated numbers in microtiter wells in RPMI 1640 supplemented with 10% fetal calf serum and L-glutamine. The EBV transformed cell line JY was used as an allogeneic stimulator cell. To prevent cell division of the JY cells, they were irradiated at 10,000 rad prior to culture. Peptides were dissolved at 50 mg/ml in DMSO and added where indicated at a final concentration of 100 μg/ml. After 6 days, cultures were tested for lysis of ^{51}Cr-labeled HLA-A2 transfected C1R cells. Wells were considered positive if lysis was > 10% above spontaneous release.

Example 3: Effect of peptides on proliferation of human PBL to the mitogen Concanavalin A (Con A).

4 x 10^5 PBL from normal donors were cultured in microtiter wells with 1 μg/ml of the indicated peptide. After 24 h, wells were pulsed with 1 μCi of ^{3}H-thymidine and harvested after an additional 24 h of culture. It was found that DQ.56-80 and DR.53-77 peptides strongly block proliferation of PBL to Con A. The DQ.71-80 peptide and the DP.53-77 did not block proliferation as strongly. Inhibition was not allele specific.

Peptides prepared as in Example 1 were preincubated for 30 min with CTLs before addition of 10^3 cpm of ^{51}Cr-labelled A2+ target cells at effector:target ratios of 1:1, 2.5:1, 5:1, and 10:1. The cytotoxicity assay was then performed as described by Clayberger *et al.*, *J. Exp. Med.* (1984) **162**:1709-1714; Rice *et al.*, *Proc. Natl. Acad. Sci. USA* (1980) 77:5432-5436. Peptides were added at 100 μg/ml and the plates were harvested after 4 h. Lysis was blocked by the DQ.56-80 peptide, but not by peptides DQ.68-77, DR.53-77 and DP.53-77.

Example 4: Determination of critical residues in DQ.56-80 for blocking lysis by CD8+ CTL.

A series of overlapping 15 amino acid peptides were prepared and tested for effects on lysis as described above. The peptides employed were DQ.53-67, 56-80, 65-79, and 71-85. The DQ.65-79 and 71-85 were most effective in blocking lysis. Similar results were obtained with CTL specific for other alleles. The DQ.71-85 was difficult to synthesize, so the 65-79 was chosen for further testing.

Example 5: Effect of single amino acid changes in inhibitory effects of DQ.65-79 peptide.

Single amino acid substitutions were introduced into the DQ.65-79 peptide. PBL were tested for proliferation of Con A as described above. Replacement of the N with D at residue 72 abrogated the inhibitory effect on proliferation of PBL to Con A. Replacement of the V at position 76 with a T led to partial loss of activity. Replacement of the N with a D at residue 74 had no effect on inhibition.

A comparison was made of the similarities and differences observed between the activity of the Class I B2702.84-75/75-84 peptide and the Class II peptide DQ.65-79.

Inhibition of:	Class I peptide	Class II peptide
CTL differentiation	+	+
CTL lysis	+	+
PBL proliferation	+	+
T cell proliferation	+	+
Rat splenocyte proliferation	+	+
Characteristics of peptide:		
Requires dimerization	+	-
Induces Ca++ influx	+	-
Immunoprecipitation	+	-
Inhibits IL-2 production	+	ND
Blocks Reporter Gene Assay (Rantes Promoter)	ND	+

It is evident from the above results, that the subject peptides provide for therapeutic opportunities to inhibit CTL attack, particularly in association with implantation of allogeneic cells or tissue, particularly solid organs. The peptides do not kill the cells, so that the immune response is readily renewed, once the administration of the peptides is terminated. In addition, the subject peptides may be used in conjunction with other immunosuppressive agents, which have undesirable deleterious effects, so that lower concentrations of the agents may be employed to reduce their undesired side effects.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS:

1. A method for the modulation of CTL activity, said method comprising:

contacting a cell population comprising CTLs from a host with a polypeptide other than an intact Class II major histocompatibility complex antigen and comprising at least 8 amino acids of the α_1-domain, D-stereoisomer analogs thereof or mutated derivative thereof having fewer than 3 substitutions or deletions, said polypeptide being present in an amount sufficient to modulate the CTL activity against allogeneic cells;

whereby said CTL activity is modulated.

2. A method according to Claim 1, wherein said cell population is present in blood.

3. A method according to Claim 1, wherein said cell population is associated with a solid organ.

4. A method according to Claim 1, wherein said contacting is *ex vivo*.

5. A method for the modulation of CTL activity, said method comprising:

contacting a cell population comprising CTLs from a host with a polypeptide other than an intact Class II major histocompatibility complex antigen and comprising at least 8 amino acids of amino acids 71-80 of the α_1-domain, D-stereoisomer analogs thereof or mutated derivatives thereof having fewer than 3 substitutions, said polypeptide being present in an amount sufficient to modulate the CTL activity against allogeneic cells.

6. A method according to Claim 5, wherein said Class II major histocompatibility complex antigen is HLA DP 0101, DQ 03011, or DR 0101.

7. A method according to Claim 5, wherein said cell population is present in blood.

associated with a solid organ.

9. A method according to Claim 5, wherein said contacting is *ex vivo*.

10. A method according to Claim 5, wherein said contacting is in combination with an immunosuppressing drug.

11. A method according to Claim 10, wherein said immunosuppressing drug is a peptide having a sequence from an α_1-helix of a class I MHC antigen.

12. A method according to Claim 11, wherein said drug comprises at least one copy of B2702.75-84 peptide.

13. A polypeptide capable of modulating the activity of CTLs, said polypeptide being other than an intact Class II major histocompatibility complex antigen and comprising at least 8 amino acids of amino acids 71-80 of the α_1-domain, D-stereoisomer analogs thereof or mutated derivative thereof having fewer than 3 conservative substitutions or deletions.

14. A polypeptide according to Claim 13, joined to an immunogen.

15. A polypeptide according to Claim 13, joined to a label capable of providing a detectable signal.

16. A polypeptide according to Claim 15, wherein said label is an enzyme, fluorescer, radioisotope, chemiluminescer, an haptenic member of a specific binding pair, an antigenic member of a specific binding pair, a receptor, or a solid support.

17. Antibodies prepared in response to a polypeptide according to Claim 13 and specifically binding to said at least 8 amino acids.

monoclonal antibodies.

19. A method for evaluating the activity of agents in immunomodulating CTLs, said method comprising:

combining under binding conditions said agent with a polypeptide according to Claim 15 and the CTL reciprocal binding member or monoclonal antibody for said polypeptide and determining the amount of binding of said polypeptide to said CTL reciprocal binding member or anti-polypeptide monoclonal antibody as compared to the binding of said polypeptide under the same conditions in the absence of said agent.

20. A method of inhibiting lysis of target cells by CTL in a mixture of cells comprising said target cells and said CTL cells, where in the absence of an inhibitor said CTL lyse said target cells, said method comprising:

adding to said mixture a lysis inhibiting amount of a polypeptide comprising at least 8 amino acids of the amino acids 71-80 of the Class II major histocompatibility complex antigen HLA DP 0101, DQ 03011, or DR 0101.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07673

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/00, C07K 14/74
US CL :424/185.1; 530/350, 395, 868
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/185.1; 530/350, 395, 868

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Examiner's major histocompatibility file references.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Automated Patent System, DIALOG biochem file: key words major histocompatibility, class II, alpha(w2)domain, T-cell.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,130,295 (SHARMA ET AL.) 14 July 1992, see abstract.	1-13, 20
A	US, A, 4,478,823 (SANDERSON) 23 October 1984, see claims.	1-13, 20
A	Critical Reviews in Immunology, Volume 11, No. 5, issued 1992, J. C. Gorga, "Structural analysis of Class II major histocompatibility complex proteins", pages 305-335, see entire document.	1-13, 20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
18 SEPTEMBER 1995

Date of mailing of the international search report
12 OCT 1995

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Inte...ational application No.
PCT/US95/07673

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proc. Nat'l Acad. Sci. (USA), Volume 90, issued February 1992, B. Nag et al., "Stimulation of T cells by antigenic peptide complexed with isolated chains of major histocompatibility complex class II molecules", pages 1604-1608, see entire document.	1-13, 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07673

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-13 and 20

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07673

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-13 and 20, drawn to Class II MHC peptides and their use in modulating CTL activity.

II. Claims 14 and 17-18, drawn to a peptide joined to an immunogen and to antibodies directed thereto.

III. Claims 15-16 and 19, drawn to labeled polypeptides and their use in assays for evaluating immunomodulating activity.

Under Unity of Invention, if multiple products or uses are claimed the first mentioned category in the claims of the application and the first recited invention in each of the other categories related thereto constitute the main invention of the claims. Instantly the modulating method and the peptide of Group I constitute the main invention.

The peptide joined to an immunogen (Claim 14 of Group II) is used to raise antibodies rather than for immunomodulating CTL activity. The claims of Group II thus pertain to a de facto second use of the peptide product. At the very least, if the peptide joined to an immunogen (Claim 14 of Group II) were placed with Group I, claims 17-18 of Group II should remain separate from Group I because the peptides and antibodies do not share a common core structure (e.g. sequence) or a common property or activity.

The labeled peptides (Claims 15-16 of Group III) are used in a method of evaluating immunomodulating activity of agents (Claim19 of Group III). As such they pertain to a use different from that first recited in the claims.